

Portable biosensor-based device for mapping the risk of grape infection by *Botrytis cinerea* in the vineyard (WINBIOTOOL)

Phase 2/2021

The second phase of the project aimed at developing and optimizing the integrated portable device for the determination of infection with *Botrytis cinerea*, laccase activity and for the application concerning the management of the risk of fungal attack in the vineyards.

In order to achieve this objective, ICDVV Valea Călugărească carried out four activities:

1. Quality and quantitative assessment of grapes infected with *Botrytis cinerea*. Laccase activity analysis on infected grapes (continuation Phase 1);
2. Operational decision process of phytosanitary treatments based on monitoring of the laccase activity in the vineyards (continuation Phase 1);
3. The analysis of environment and technological factors that determine the occurrence and development of gray mould in the vineyards (continuation Phase 1);
4. Working visits, scientific events and dissemination of results.

RESULTS

1. Quality and quantitative assessment of grapes infected with *Botrytis cinerea*. Laccase activity analysis on infected grapes

The risk assessment of *Botrytis cinerea* fungus has been achieved in Valea Calugareasca viticultural centre, on 5 wine grapevine cultivars: three highly susceptible varieties to gray mould – Chardonnay, Sauvignon Blanc, Feteasca Alba, and two varieties with medium resistance – Feteasca Neagra and Cabernet Sauvignon.

Although the environmental conditions were favorable for the development of *Botrytis* starting with June, due to the fact that the phytosanitary treatments were applied at the optimal time in accordance with the annual phytosanitary control plan, the degree of attack was very low.

It recorded values between 0,04% (Feteasca neagră variety) and 2,0% (Chardonnay variety) during veraison phenophase and between 0,25% (Fetească neagră) and 1,24% (Sauvignon blanc) during full ripening of grapes.

The phenolic potential of grapes has changed significantly at harvest, depending on the degree of attack.

The degradation of polyphenolic compounds in infected must can be explained by the increasing of laccase activity in the infected must with *Botrytis* from 2,42 U/mL (Chardonnay) to 4,81 U/mL (Sauvignon blanc), at an attack intensity of 25%, at values between 15,24 (Fetească alba) and 19,92 (Sauvignon blanc) at an attack intensity of 51 – 75%.

A direct correlation was put into evidence between the degree of infection of musts with *Botrytis cinerea* and laccase activity.

As a consequence of the degradation of polyphenolic compounds and the intensification of laccase activity in the infected musts, the change of chromatic characteristics, in the sense of increasing the values of the „Hue” parameter and the yellow color of musts was highlighted, in direct correlation with the oxidative processes.

2. Operational decision process of phytosanitary treatments based on monitoring of the laccase activity in the vineyards

Operational decision process of phytosanitary treatments, carried out in the excel program, is based on the knowledge and parameterization of the elements that favor the appearance and evolution of the disease (independent variables) and of the elements that define the effects produced by them (dependent variables).

In order to assess the risk of infection, in addition to the climatic elements, were taking into account other factors involved in the appearance and evolution of gray mould, namely, the genetic predisposition of the varieties, the location, density and the general condition of the plantation, the phenophases in which the incidence of disease can be high.

This information has been included in a Database and used, through the data processing model, to establish the risk of damage of vineyards as a consequence of fungal attack.

The selection of the most suitable solutions to reduce the effect of the appearance and development of the gray mould attack is made according to a risk factor, established based on the evaluation of 12 parameters that play an important role on grapevine productivity and sustainability, with notes from 1 to 5.

These parameters quantified during the key phenophases in which the incidence of the disease can be high are: vegetation period, amount of precipitation, relative humidity of the air, period (hours) of retention of water on leaves and grapes, uniformity of precipitation distribution, average temperature during the wetting of the leaves, resistance of vinifera varieties to grey mould, data concerning grapevine plantations, laccase activity.

Note 1 represents a low risk and note 5, a very high risk of vineyards damage caused by gray mould.

Depending of the obtained results, possible interventions can be suggested, respectively, prophylactic or control measures, measures for partial restoration of the vines affected by the fungal attack, the schedule of phytosanitary treatments, indicating of the moment of application.

3. The analysis of environment and technological factors that determine the occurrence and development of gray mould in the vineyards

For establishing the influence of environment and technical conditions which determine the occurrence and development of gray mould (*Botrytis cinerea*) in the vineyards, two grapevine varieties were studied: Cabernet Sauvignon with medium resistance to Botrytis infection, and Chardonnay with high susceptibility.

The ecoclimatic conditions during the vegetation period of the grapevine in 2021 were characterized by a reduced heliothermal regime and moderate water resources, with multiple influences in the evolution of vegetative phenophases.

Taking into account the average temperature during the wetting of the foliage and the duration of the leaf moisture, the amount of precipitation and the value of relative humidity, favorable conditions for the manifestation of gray mould were in June (95,4 hours) and August (68,8 hours), the period in which the temperature had optimal values for the development of Botrytis, 19.4°C and 21.4°C respectively.

Analysing the evolution of soil moisture at a depth of 0-100 cm during the vegetation period of the grapevine it was found that, at the beginning of the vegetation period, April, the soil moisture was at a normal level, increased during July, due to heavy rainfall, after which decreased constantly until the end of the growing season, reaching values close to the wilting coefficient of the plant during September.

According to the Risk indices established in Phase 1, during the Budburst there was a low or moderate risk of infection, and during the others phenopases a moderate or high risk of infection.

Although the environmental conditions were favorable for the development of *Botrytis* starting with June, due to the phytosanitary treatments applied the degree of attack recorded very low values, between 0.95% at Chardonnay and 0.52% at Cabernet Sauvignon. Following the attack of *Botrytis cinerea*, the production losses were only 2.2-3.5%.

4. Working visits, scientific events and dissemination of results

PROJECT MEETINGS

During 2021, frequent working visits were held between Romanian partners at ICDVV Valea Călugărească with the aim of:

- Establishing the elements that must be taken into account for the development of the electrochemical immunosensor;
- Selection of grapes and musts samples with different degree of infection with *Botrytis cinerea* which were obtained by artificial infection from ICDVV Valea Călugărească;
- Preparation of spore samples developed on the mycelium of some strains of gray mould in order to use them for the development of the electrochemical immunosensor;
- Testing the electrochemical method for laccase activity in comparison with spectrophotometric method.

Online "Kick-off meeting" of WINBIOTOOL via Microsoft teams was organized on 25th May by ENKOA SYSTEM, S.L. coordinator of project, during which an analysis concerning the accomplishment of the activity carried out in 2020 was made, and it was established the activities planned for 2021.

DISSEMINATION OF RESULTS

The results obtained within the project were disseminated by participating in two scientific events, organized online:

- 1) Advances in Food Chemistry 2021 Conference (AdFoodChem 2021), April 15 – 17th, with the paper "Screen printed electrochemical sensors for monitoring laccase and lysozyme during wine production", authors: Vasilescu, A., Gáspár S., Titoiu. A.M., Porumb R., Brîndușe E. (oral presentation);
- 2) International Symposium ISB-INMA TEH' 2021 Technologies and Technical Systems in Agriculture, Food Industry and Environment, 29th October, with the paper "Quality and quantitative assessment of grapes infected with *Botrytis cinerea* in vineyards", authors: Ion Marian, Brîndușe Elena, Nedelcu Cătălina Loredana (poster presentation).